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(57) Abstract

A therapeutic agent capable of binding to the F'-G' loop of domain 4 of the common β_c chain of the receptor for GM-CSF, IL-3 and IL-5. The amino acid Tyr^{421} which is located in the F'-G' loop is critical in the high affinity binding and stimulation of function of GM-CSF, IL-3 and IL-5 to the common β_c chain of their receptors. Other receptors for cytokines also show hydrophobic amino acids in analogous positions and it is probable that they too will play a pivotal role in binding of respective cytokines to them and in modulating function. Agents capable of binding to the F'-G' loops are suggested and should be a therapeutic value.

CYTOKINE ANTAGONISTS AND AGONISTS

FIELD OF THE INVENTION

This invention relates to antagonists and agonists of cytokines, the therapeutic use of such antagonists and agonists, and method of isolation of such antagonists and agonists.

BACKGROUND OF THE INVENTION.

Human granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukins IL-3 and IL-5 are cytokines involved in hemopoietic cell survival, production and function (reviewed in Lopez *et al*, 1992). Because of these properties, IL-3 and GM-CSF are currently being used clinically for bone marrow reconstitution following chemotherapy and radiotherapy (Groopman *et al*, 1987). However, it is also becoming apparent that excessive or aberrant production of GM-CSF, IL-3 and IL-5 can lead to disease states. For example, elevated amounts of GM-CSF have been found in the lungs of allergic individuals (Kato *et al*, 1992) and in the joints of patients with rheumatoid arthritis (Williamson *et al*, 1988). Elevated mRNA for IL-3, GM-CSF and IL-5 have been found in the skin of allergic individuals (Kay *et al*, 1991). GM-CSF can stimulate the proliferation of leukaemic cells (Young *et al*, 1986), and IL-3 has been shown to be produced by follicular B cell lymphomas in an autocrine fashion resulting in the proliferation of these cells in an IL-3-dependent manner (Clayberger *et al*, 1991).

It is clear from these clinical situations that antagonising GM-CSF, IL-3 and IL-5 can be of therapeutic value, and depending on the condition in question, antagonising one of these cytokines may be sufficient.

A number of suggestions for antagonists have already been made, for example in specification PCT/AU89/00177 and in specification PCT/AU94/00432 variants of GM-CSF are identified as antagonists to GM-CSF action however there is no indication that these antagonists are effective for more than only GM-CSF action.

However, in other situations the simultaneous antagonism of all three GM-CSF, IL-3 and IL-5 may be desirable or indeed necessary. For example, eosinophils which are believed to be the major cell type involved in allergy can be maintained in numbers and be stimulated by either IL-3, GM-CSF or IL-5 (Lopez *et al*, 1989). Antagonism of all three cytokines may thus be necessary to inhibit the actions of eosinophils and basophils. Similarly, basophils which are also believed to play an effector role in

allergy can be stimulated by either IL-3, GM-CSF or IL-5 (Lopez *et al.*, 1990) Antagonism of GM-CSF, IL-3 and IL-3 may be accomplished by the concomitant administration of specific antagonists for each different cytokine. Though feasible, this approach has the disadvantage of having to administer up to three different proteins which is not only inconvenient but which also increases the risk of immunogenicity and other side-effects.

One condition which is prevalent that may be exacerbated by elevated levels of these three cytokines is asthma. The roles of GM-CSF, IL-3 and IL-5 in asthma and allergy have been and continue to be extensively studied. Several studies have shown, by *in situ* hybridisation, increased levels of IL-5 mRNA in lung mononuclear cells (Fukuda *et al.*, 1994; Robinson *et al.*, 1992; Marini *et al.*, 1992; Hamid *et al.*, 1991) and in eosinophils (Broide *et al.*, 1992) in asthmatic patients. Immunochemistry has also revealed increased amount of IL-5 protein in these tissues (Ackerman *et al.*, 1994). In allergen-induced late-phase cutaneous reactions in atopic subjects increased mRNA for IL-5, IL-3 and GM-CSF have been noted (Kay *et al.*, 1991).

Bronchoalveolar lavage (BAL) fluids from symptomatic asthma patients had greater IL-5 levels than patients with asymptomatic asthma (Sur *et al.*, 1995). Furthermore when symptomatic asthma patients were challenged with antigen in one lung segment, significant levels of IL-5 were noted (980pg/ml) compared with sham challenged segment of the same patient (2.8pg/ml) (Sur *et al*, 1995). In another study with similar design IL-5 levels increased from undetectable to 2800pg/ml in both allergic and non-allergic asthmatics (Zangrilli *et al.*, 1995). A causal relationship between levels of IL-5 and asthma is suggested from studies in which treatment of moderately severe asthmatic patients with corticosteroids for two weeks resulted in a reduction of the number of cells expressing IL-5 mRNA (Bentley *et al.*, 1996). This reduction was correlated with clinical improvement and also with loss of $CD3^+$ T cells and activated ($EG2^+$) eosinophils.

GM-CSF has also been detected in the lungs of asthmatics. Indeed in one study of sputum cytokines, GM-CSF appeared to have the dominant effect on eosinophil survival (Adachi *et al.*, 1995).

IL-5 also activates eosinophils to express the EG2 epitope. In several studies increased IL-5 levels were associated with $EG2^+$ eosinophils in the lung (Fukuda *et al.*, 1994; Bentley *et al.*, 1996). Furthermore there is evidence of a tissue-specific activation of eosinophils. In one experiment, activation of eosinophils in the lung was compared to those in the blood of the same patient. Activation was assessed by cell surface

expression of CR-3, p150/95, CD67, CD63 and loss of L-selectin. In patients challenged with endobronchial antigen both peripheral and lung eosinophila were seen at 24h, however only lung eosinophils had increased levels of GM-CSF mRNA suggesting a local activation of these cells. In addition, there was evidence of activation in lung-derived eosinophils but not those from blood. This result is compatible with a specific effect of IL-5 on lung eosinophils and their involvement in asthma.

Animal models also suggest a role for IL-5 in asthma. The most significant data exists with an antibody (TRFK-5) against IL-5 in monkey models of asthma (Mauser *et al.*, 1995). Ascaris-sensitive cynomolgus monkeys were challenged with aerosolised *Ascaris suum* extracts. 0.3mg/kg TRFK abolished increased airway reactivity and diminished eosinophil numbers in BAL fluids. Interestingly this inhibition persisted for 3 months (Mauser *et al.*, 1995). Guinea pig studies supported this conclusion (Mauser *et al.*, 1993). Furthermore mice in which the IL-5 gene was genetically ablated not only had no detectable IL-5 and significant reduction in eosinophil numbers but also developed significantly less severe asthma (as manifest by airways hyperreactivity and lung damage) than IL-5 gene positive litter-mates (Foster *et al.*, 1996). A clear example of restoration of airway responsiveness to methacholine was seen after IL-5 deficient mice were given IL-5 expressing-, but not control-, vaccinia virus infections (Foster *et al.*, 1996).

A likely role for GM-CSF in asthma has also been suggested by overexpression of GM-CSF in rat lung leading to eosinophilia, macrophage granuloma and fibrotic reaction, a triad also seen in asthma (Xing *et al* 1996)

Human interleukin (IL)-3, granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-5 exert their biological effect by binding to specific surface receptors on the surface of cells (Bagley *et al*, 1995; Elliott *et al*, 1989; Park *et al*, 1989; Lopez *et al*, 1991). The receptors are heterodimers comprising an α chain which is specific for each ligand, and a β chain (β_c) which is shared between the three receptors (Lopez *et al*, 1992; Kitamura *et al*, 1991). Whilst each ligand binds to the respective α chain, β_c provides high affinity binding and allows signalling (Miyajima *et al*, 1992 and US Patent 5112961 by Hayashida *et al*)). The inventors in US patent no 5112961 suggest that the high affinity receptor is a valuable tool for screening candidate GM-CSF agonists and antagonists.

Because all three of these cytokines act through a common receptor subunit (β_c) we previously hypothesised (Bagley *et al*, 1995, which reference is incorporated herein), it may be possible to simultaneously inhibit the action of GM-CSF, IL-3 and IL-5 with a

single compound. There has, however, to date been no means whereby to approach the problem of finding an effector that acts co-ordinately on all three cytokines. It is not clear, for example, what region of the common receptor subunit (β_c) is being bound by these cytokines, and whether the same region is bound for all three cytokines or whether three different regions are involved.

Analysis of the β_c has shown that it contains four extracellular domains (Goodall *et al*, 1993) with domain 1 representing the most N-terminal domain and domain 4 representing the most membrane proximal domain. Each domain consists of seven β strands linked together by intervening loops. The loop of interest, the F'-G' loop, is located in domain 4 of β_c. The residues of interest are numbered from the N-terminus at primary translation terminus with the initiation methionine being Met^1.

The molecular basis for the affinity conversion of β_c to each ligand is not fully understood as the ligand-receptor complex has not yet been crystallised. However, by analogy to the interaction of growth hormone (GH) with its homodimeric receptor (DeVos *et al*, 1992) there is likely to be direct interaction between ligand and receptor α and β chains. In the GH:GH receptor system the contact points between GH and GHbp2 have been determined from X-ray crystallography of the GH:GHbp1 and 2 complex (DeVos *et al*, 1992). By analogy with this system we (Woodcock *et al*, 1994) and others (Lock *et al*, 1994) have previously shown that the B'-C' loop of β_c plays some role in high affinity binding of GM-CSF, IL-3 and IL-5. Specifically it has been shown that three residues, Tyr^{365}, His^{367} and Ile^{368} are important for GM-CSF and IL-5 high affinity binding but are only marginally involved in IL-3 high affinity binding (Woodcock *et al*, 1994). These results imply that targeting these three amino acids with appropriate compounds may impair GM-CSF and IL-5 but not IL-3-mediated activity.

SUMMARY OF THE INVENTION

Using molecular modelling techniques we have recently postulated that the F'-G' loop may be involved in ligand binding (Bagley *et al*, 1995). The invention arises out of an investigation on this predicted F'-G' loop of β_c and from the finding that this loop is essential for the high affinity binding and signalling of all three cytokines GM-CSF, IL3 and IL-5.

A further aspect of this invention arises as a result of the surprising identification of a single amino acid in the receptor β_c chain for GM-CSF, IL-3 and IL-5 which is necessary for the high affinity binding of all of these three ligands to their surface receptors and for signalling and the fact that all three of these cytokines require the F-

G' loop for high affinity conversion. It is believed that compounds that bind to Tyr^{421} or inhibit the binding of ligand to Tyr^{421} will behave as generic antagonists of IL-3, GM-CSF and IL-5. It is thought that compounds that bind to the F' - G' loop will sterically inhibit this binding.

Additionally this finding is likely to have implications for other members of the cytokine receptor superfamily some of which are shared subunits in a given subfamily (that is they bind several cytokines), and some which are ligand specific and bind to only one cytokine. The receptor α-chains for GM-CSF, IL-3 and IL-5 and β_c belong to the rapidly expanding cytokine receptor superfamily. Within this superfamily several sub-families are now emerging that are characterized by the sharing of a communal receptor subunit by multiple ligands: gp130 acts as an affinity converter and signal transducer for IL-6 (Hibi *et al.*, 1990; Taga *et al.*, 1992), IL-11 (Hilton *et al.*, 1994), oncostatin M (Liu *et al.*, 1992), ciliary neurotrophic factor, leukaemia inhibitory factor (LIF) (Ip *et al.*, 1992) and cardiotrophin-1(Pennica *et al.*, 1995); the LIF receptor (LIFR) also binds ciliary neurotrophic factor (Davis *et al.*, 1993), cardiotrophin-1 (Pennica *et al.*, 1995) and oncostatin M in addition to LIF (Gearing *et al.*, 1994); IL-2R β supports affinity conversion and signalling of IL-2 and IL-15 (Giri *et al.*, 1994); IL-2R γ chain affinity converts IL-2 (Takeshita *et al.*, 1992), IL-4 (Russell *et al.*, 1993), IL-7 (Noguchi *et al.*, 1993), IL-9 (Kimura *et al.*, 1995)and IL-15 (Giri *et al.*, 1994); evidence also suggests that IL-4 and IL-13 share a receptor component (Zurawski *et al.*, 1993) and this subunit has recently been cloned (Hilton *et al.*, 1996). It is not known which residues in gp130, LIFR and IL-2R β and γ chains are important for ligand binding or indeed whether different ligands share or have unique sets of binding determinants on these communal receptor subunits. Because these common subunits are vital for transducing signals by several ligands, the possibility arises that interfering with the ability of these common subunits to bind ligand or to form homodimers may affect the action of more than one ligand.

An alignment of the predicted F'-G' loop of β_c with that of several common as well as ligand-specific receptor subunits revealed the presence of tyrosine and other similarly hydrophobic residues in this region (Table 3). Although the exact length of this putative loop varies between 7-14 amino acids amongst the different receptors, it is constrained by two strongly conserved sequences; the VRVR consensus at the N terminus and the WSXWS consensus at the C terminus. It is suggested that although the nature of the ligand-receptor interaction will be specific to the system involved, the hydrophobic aromatic residues in the receptor represent strong candidates for ligand interaction. Thus members of the cytokine receptor family may use a structurally conserved receptor framework to display a series of loops that through predominantly

hydrophobic interactions support the binding of specific ligands. Support for this notion also stems from mutational analysis of the IL-6R which showed that a mutant in which both Glu^{297} and Phe^{298} in the F'-G' loop were substituted exhibited loss of IL-6 binding (Yawata *et al.*, 1993).

A recent analysis of the residues involved in binding growth hormone in GHR revealed that the greatest contribution to affinity came from hydrophobic interactions (Clackson and Wells, 1995). Systematic alanine substitution of the 33 residues in GHR known to be inaccessible to solvent upon growth hormone binding showed that substitution of only eleven of these residues had any significant effect on affinity and, of these, six were hydrophobic. Furthermore these hydrophobic residues are clustered on the surface of GHR in a region which forms the interface for interaction with growth hormone. In addition, in GHR the greatest reduction in affinity occurred on substituting two tryptophan residues (Clackson and Wells, 1995; Bass *et al.*, 1991) implicating hydrophobic aromatic residues as being very important for ligand interaction. From our present study and our previous work (Woodcock *et al.*, 1994) we have now identified a total of three hydrophobic residues in predicted loops which play a role in ligand interaction in β_c, Tyr^{365}, Ile^{368} and Tyr^{421}. Thus it appears that binding of β_c to GM-CSF, IL-3 and IL-5 is also supported predominantly by hydrophobic interaction.

It is interesting to compare these findings with β_c of GM-CSF, IL-3 and IL-5 receptors with the crystal structure and mutational analysis of the GH-GHR. In contrast to the heterodimeric nature of the GM-CSF, IL-3 and IL-5 receptors, the GHR is a homodimer. The two identical subunits of GHR bind growth hormone sequentially and in a non-equivalent manner. In the crystal structure, the two growth hormone-binding proteins are correspondingly identified as GHbpI and GHbpII. The initial interaction of GHbpI utilises sites I contacts in growth hormone and is relatively strong with a dissociation constant of 600 pM (Cunningham *et al.*, 1991). Subsequent interaction with GHbpII through site II contacts decreases the dissociation constant to 200 pM. Therefore, interaction of GHbpII with growth hormone bound to GHbpI confers a 3-fold increase in affinity on the receptor complex. Thus, functionally, human β_c is analogous to GHbpII, binding only after initial association of ligand with receptor α-chain and conferring up to 1000-fold increase in affinity on the receptor complex (as in the case of IL-3). The results presented here, however, show three significant differences from the GH-GHR interaction. First, the F'-G' loop in GHbpI and not in GHbpII (analogous to β_c) is involved in ligand binding. Second, although residues in the F'-G' loop of GHbpI are effectively solvent excluded on binding of

growth hormone, indicating close contact between ligand and receptor, there is seemingly very little productive interaction between the two proteins in this region. Alanine-scanning mutagenesis across residues of this loop in GHR had very little effect on growth hormone binding affinity relative to wild type receptor (Clackson and Wells, 1995; Bass *et al* ., 1991). Hence the contact between the F'-G' loop of GHbpI and growth hormone contributes little to the binding affinity of the receptor. This is in contrast to our findings here, where we observe complete loss of affinity conversion for all ligands on substitution of Tyr^{421}, indicating a very strong interaction between this residue and ligand in the wild type molecule. Third and consistent with the lack of productive interaction between the F'-G' loop of GHR and GH, there are no hydrophobic residues in this loop. Thus despite GHR using contact sites of a clearly hydrophobic nature, these lie elsewhere in this receptor, indicating that the contribution made to affinity by the various contact sites is different in β_c relative to GHR.

In a first broad aspect the invention could therefore be said to reside in a therapeutic agent capable of binding to the F' - G' loop of domain 4 of the common β_c chain of the receptor for GM-CSF, IL-3 and IL-5, or to an analogous loop of another cytokine receptor the binding of which agent has an antagonist or agonist effect on the respective cytokine or one of the cytokines.

In a first form of the first aspect the cytokine receptor is a common signal transducing receptor, which may be one selected from the group comprising β_c, gp130, LIFR, IL-2Rβ IL-2Rγ, and IL-4R/IL-13R. In a more specific form of this aspect the receptor is β_c. In a preferred form of this aspect the agent has the antagonist or agonist effect on all respective cytokines that bind to the receptor.

In a second form of the first aspect of the invention the F'-G' loop includes a hydrophobic amino acid. The aromatic hydrophobic amino acid for the respective receptor may be as indicated in Table 3 and the invention may be restricted to the receptor listed therein, or may be to a tyrosine and the invention may be restricted to receptors with a tyrosine present in the F'-G' loop. The receptor may alternatively be restricted to those receptors that also interact with β_c.

In a third form of the first aspect of the invention the cytokine receptor is a signalling receptor, which in one more specific form is specific for a single cytokine, and may be selected from the group comprising EPOR, TPOR and OBR.

As indicated above in the more specific form of the first form of the first aspect of the invention the common signal transducing receptor is β_c. The amino acid specificity at position 421 is quite stringent and the therapeutic agent may need to be identified as requiring the presence of Tyr^{421} for binding, or of sterically hindering the normal interaction that Tyr^{421} has. The agent may be capable of alleviating the symptoms of asthma.

The therapeutic agent may take the form of any one of a number of classes of compounds and may be selected from a group comprising, antibodies, peptides, oligosaccharides, oligonucleotides, or other organic or inorganic compounds.

In a second aspect the invention could be said to reside in a method of isolating a therapeutic agent capable of binding to the F' - G' loop of domain 4 of the common β_c chain of the receptor for GM-CSF, IL-3 and IL-5, or to an analogous loop of another cytokine receptor, said method including the steps of screening candidate molecules for their capacity to bind to the said loop. In one form of this second aspect of the invention the step of screening candidate molecules for their capacity to bind to said loop, comprises screening for the capacity to bind to a sequence selected from the group comprising SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11 and SEQ ID NO. 12. It will also be understood that as an alternative only the loop to be screened may be selected from those derived from a common signal transducing receptor, or as a second alternative from those receptors having a hydrophobic amino acid residue, or as a third alternative from those receptors that are signal transducing receptors.

Alternative the invention could be said to reside in a method of isolating a therapeutic agent, by screening for the ability of compounds to bind to Tyr^{421} of the common β_c chain of the receptor for GM-CSF, IL-3 and IL-5, or the corresponding aromatic hydrophobic residue of gp130 or to an analogous aromatic hydrophobic residue of another common signal transducing receptor or of a specific receptor responsive to other cytokines.

The agent may be an antibody or fragment thereof and the method of isolating such an antibody will be understood to further including the steps of inoculating an animal with a peptide molecule having the F'-G' loop, fusing antibody producing cells with a myeloma cell line and screening for a cell line that produces an antibody reactive with said F'-G' loop, and harvesting antibodies from said cell line, testing for inhibition of high affinity binding and testing for inhibition or excitation of function. This may

further include making small fragments of antibodies produced by the said cell line capable of binding said the loop. The cell line may conveniently be a mouse cell line and the method may include the further step of humanising the said antibody fragments by replacing mouse sequences with human sequences in the non-binding regions.

In a third aspect the invention could be said to reside in a method of treating an human or an animal for a condition, said method including the step of administering a therapeutic agent as identified above in a pharmaceutically acceptable carrier and in a therapeutically effective dose.

It may be desired to treat the condition with one or more of the therapeutic agents identified or defined herein in combination, and perhaps in combination with other therapeutic agents.

The treatment may be aimed at being preventative by reducing the risk of contracting the condition, or the treatment may be used to alleviate or obviate the condition. The administration of the therapeutic agent can be any pharmaceutically acceptable form and in a suitable carrier.

It is thought that the construction of compounds that bind the F' - G' loop of β_c will be therapeutically useful for intervention in conditions where IL-3, GM-CSF and IL-5 play a pathogenic role, mainly allergy, asthma, leukaemia, lymphoma and inflammation including arthritis.

Similarly for other cytokine receptors it is thought that antagonists or agonists will be therapeutically useful. Thus for common signal transducing receptors. Since gp130 is functionally analogous to β_c in the GM-CSF/IL-3/IL-5 receptor system, in that it is a common binding sub-unit and signal transducer for the IL-6, oncostatin M (OSM), ciliary neurotrophic factor (CNTF), leukaemia inhibitory factor (LIF) and IL-11, it is suggested that targeting/blocking of this tyrosine will lead to antagonism of the IL-6, LIF, OSM, CNTF and IL-11. Antagonism of this receptor system will be useful in inflammation, leukaemia and lymphoma. Antagonists of IL-2Rβ/γ may be useful as immunosuppressants. Antagonists of LIFR may be useful for the prevention of implantation of embryos in uteri. Antagonist of IL-4/IL-13 will inhibit IgE production and may be useful in treating asthma and allergies.

For specific subunits. Antagonist of IL-3 may be useful in treating allergy and follicular B cell lymphoma. Antagonists of IL-4 may inhibit IgE production, and be useful for treatment of asthma and allergy. Antagonists of IL-6R may be useful as an

anti-inflammatory and may be used to inhibit myeloma growth. Antagonists against IL-7 may be useful as an immunosuppressant. Antagonists of the leptin receptor (OBR) may be useful in the treatment of cachexia, weight loss in conditions such as AIDS, cancer, and parasitic diseases.

It can be seen that an antagonist effect has been found, however, it is to be understood that the invention may also encompass agonists. The residue identified clearly plays a pivotal role in the action of the system and whilst some molecules that interact with the F' -G' loop of β_c or corresponding residues in other receptors (such as shown in table 3) can be expected to have an antagonist effect it is equally possible that they have an agonist effect particularly in receptors that can be activated by homodimerization.

Firstly the common signal transducing receptors. Agonists agents that bind to β_c may be used to stimulate hemopoiesis, and to boost immune response against microorganisms. Agonists agents that bind to LIFR may be useful in the suppression of embryonic stem cell differentiation. Agonists agents that bind to IL-2Rβ may be used in immunostimulation. Agonists agents that bind to IL-4R/IL-13 may have anti-tumour activity.

Turning now to specific subunits. Agonists agents that bind IL-3R may be use in the *in vivo* and *ex vivo* expansion of early hemopoietic cells. Agonists agents that bind to IL-4R may have useful anti-tumour activity. Agonists agents that bind to IL-7R may have useful anti-tumour immunity. Agonists agents that bind IL-11 may prove a useful adjunct to cancer therapy. Agonists agents that bind to EPOR may be used to correct anemia of chronic renal failure, of chronic inflammatory diseases and of malignant diseases. Agonists agents that bind to TPOR, may be useful for correcting thrombocytopenia (such as may be associated with chronic inflammatory diseases, malignancies, chemo- and radio- therapy).

Examples of useful agonists are those for erythropoietin and thrombopoietin to elevate erythrocyte and platelet numbers in blood following blood cell loss, chemotherapy, radiotherapy, immunosuppression or bone marrow transplantation. Agonists of OBR may be used to induce weight loss, and in particular for obesity which is considered to be a contributing factor of hypertension, coronary heart disease and non insulin-dependent diabetes mellitus. The molecules whether agonist or antagonist can be isolated on the basis of their ability to interact with the identified residue.

By way of a shorthand notation the following three and one letter abbreviations for amino acid residues are used in the specification as defined in Table 1.

Where a specific amino acid residue is referred to by its position in the polypeptide of an protein, the amino acid abbreviation is used with the residue number given in superscript (i.e. Xaa^n)

TABLE 1

Amino Acid	Three-letter Abbreviation	One letter Abbreviation
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic Acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A Is a schematic representation of the extracellular domain of human β_c showing two cytokine receptor modules (CRM1 & 2) (Goodall *et al.*, 1993) and conserved features of the cytokine receptor superfamily (Bazan 1990).

Figure 1B Represents the amino acid sequences corresponding to the putative F'-G' loop in the membrane proximal CRM2 of β_c (Hayashida *et al.*, 1990) aligned with the F'-G' loops of growth hormone receptor (GHR) (Leung *et al.*, 1987) and prolactin receptor (PRLR) (Boutin *et al.*, 1988). Conserved structural motifs are boxed and previously identified residues that are involved in ligand (GH) contact (De Vos *et al.*, 1992, and Somers *et al.*, 1994) are shown in bold. Mutant forms of β_c which were made to identify ligand binding determinants are shown with mutated residues in bold.

Figure 2 Are graphic representations showing that alanine substitution of residues 418-422 in β_c abolishes high affinity GM-CSF and IL-3 binding. Scatchard transformation of saturation binding experiments using ^{125}I-GM-CSF (left panel) and ^{125}I-IL-3 (right panel) were carried out on COS cells expressing both GM-CSFR and IL-3R α-chains together with wild type (o) or mutant ^{418}AAAAA^{422} (●) β_c. The radio-iodinated ligand concentration range for both GM-CSF and IL-3 was 10 pM-10 nM and non-specific binding was determined in the presence of 1 μM unlabelled ligand. The broken line indicates the high and low affinity binding components using wild type β_c, and the solid line represents the line of best fit for the ^{418}AAAAA^{422} mutant β_c as determined using the LIGAND program. A representative experiment is shown and the K_d values derived from these and several other experiments are shown in Table 2.

Figure 3 Shows the effect of individual alanine substitution of residues Arg^{418}, Thr^{419}, Gly^{420}, Tyr^{421} and Asp^{422} of human β_c on high affinity GM-CSF and IL-3 binding. Scatchard transformation of saturation binding studies with ^{125}I-GM-CSF (top panel) and ^{125}I-IL-3 (bottom panel) were carried out as described in Figure2. The broken line indicates the high and low affinity binding components using wild type β_c (o), and the solid line represents the line of best fit for each mutant β_c (●) as determined using the LIGAND program. A representative experiment is shown and the K_d values derived from these and similar studies are shown in Table 2.

Figure 4 Shows the effect of re-introducing Tyr^{421} into a poly-alanine substituted β_c (^{418}AAAYA^{422}) on high affinity GM-CSF and IL-3 binding.

Scatchard transformation of saturation binding studies with ^{125}I-GM-CSF (left panel) and ^{125}I-IL-3 (right panel) were carried out as described in Figure 2. The broken line indicates the high and low affinity binding components using wild type β_c (o), and the solid line represents the line of best fit for the ^{418}AAAYA^{422} mutant β_c (●) as determined using the LIGAND program. A representative experiment is shown and the K_d values derived from these and similar studies are shown in Table 2.

Figure 5A Is a representation of an autoradiogram showing that STAT5-like (STAT5-L) activity is induced by GM-CSF and IL-3 only in Jurkat cells expressing appropriate α-chains together with wild type (Wt) β_c. Nuclear extracts were prepared from transfected Jurkat cells stimulated for 1 hr with or without 300 nM cytokine and analysed by electrophoretic mobility shift assay using a STAT5 specific probe.

Figure 5B Is a representation of an autoradiogram similar to that of Figure 5A showing that the DNA binding protein induced by cytokine in receptor transfected Jurkat cells behaves like STAT5. Nuclear extract prepared from GM-CSF-stimulated transfected Jurkat cells was subjected to electrophoretic mobility shift assay in the presence of 50-fold molar excess of competing non-radioactive oligonucleotides. The oligonucleotides used for competition were: the β-casein promoter element (β-cas), a mutant of the β-casein promoter element (mut β-cas), an AP-1 consensus binding site (AP-1) and the haemopoeitin receptor response element (HRRE).

Figure 6A Is a graphical representation that Y421A β_c activates STAT5-L with reduced potency in response to GM-CSF stimulation. A dose-response study of cytokine-induced STAT5-L activity was carried out on Jurkat cells transfected with α-chains and either wild type β_c (o), or Y421A (●). STAT5-L activity was determined by electrophoretic mobility shift assay using a STAT5 specific probe and subsequently quantified by phosphorimager analysis. The results are expressed as a percentage of maximal activity obtained after correction for background and protein concentration.

Figure 6B Is a graphical representation that Y421A β_c activates STAT5-L with reduced potency in response to IL-3 stimulation. The experimentation and procedures are as in figure 6A.

Figure 7 A model of the spacial arrangement between the putative B'-C' and F'-G' loops of β_c and the first α helix of GM-CSF, IL-3 or IL-5 based on the GH-GHR crystal structure. Only the membrane proximal domains of β_c (CRM2) and the first α helix (helix A) of GM-CSF, IL-3 or IL-5 are shown, with Tyr^{365} and Tyr^{421} of β_c and the conserved Glu of GM-CSF, IL-3 or IL-5 shown in space-filling representation.

DETAILED DESCRIPTION OF THE EXAMPLES

EXAMPLE 1

Demonstrating the efficacy of Tyr^{421} in β_c subunit binding

Rationale for mutagenesis of putative loops in the human β chain.

The region of β_c we have targeted for mutagenesis is flanked by regions that show conservation throughout the cytokine receptor superfamily: the VRXR consensus described previously (Patthy 1990) where X most commonly represents an aliphatic residue, and the WSXWS motif (Cosman *et al.*, 1990; Bazan 1990) (Figure 1). The WSXWS motif has long been recognised as a characteristic feature of this receptor family and several studies have been directed towards understanding its role in receptor function (Miyazaki *et al.*, 1991; Yoshimura *et al.*, 1992; Quelle *et al.*, 1992; Rozakis-Adcock and Kelly 1992; Baumgartner *et al.*, 1994). The GH-GHR crystal structure (De Vos *et al.*, 1992) and more recently the crystal structure of GH bound to prolactin receptor (Somers *et al.*, 1994) gives us some insight into the structure of the VRXR-WSXWS region in cytokine receptors in general. The two β strands concerned interact exquisitely to form a stack of aromatic side-chains interleaved by the hydrophobic portions of the basic side-chains and as a consequence the amino acids separating these two motifs form a loop. Residues in this loop are solvent inaccessible in the ligand-bound complex in both resolved receptors (De Vos *et al.*, 1992; Somers *et al.*, 1994). We have therefore concentrated our attention here on the analogous F'-G' loop region in the membrane proximal cytokine receptor module (CRM) of β_c to determine its role in GM-CSF, IL-3 and IL-5 binding.

Identifying a region involved in GM-CSF, IL-3 and IL-5 high affinity binding.

To investigate the possible involvement of the putative F'-G' loop of β_c in high affinity receptor complex formation, the region was disrupted by substitution of alanines across residues 418-422 (Figure 1). The ability of this n-alanine substituted mutant β_c (^{418}AAAAA^{422}) to support high affinity ligand binding was studied by co-expressing it with both GM-CSFR and IL-3R α-chains on COS cells and performing radioligand saturation binding studies. Routinely COS cells were electroporated with both α-chain cDNAs together with mutant β_c cDNA so that the same transfectant could be analysed for both GM-CSF and IL-3 binding. In the absence of transfected β_c, GM-CSFR and IL-3R α-chain transfected COS cells bound GM-CSF and IL-3 with low affinity only (Table 2). Co-expression of wild type β_c conferred high affinity binding on GM-CSF and IL-3 but as the α-chains were expressed in excess of β_c, low affinity binding was still in evidence as represented by the curvilinearity of the corresponding Scatchard plots (Figure 2, Table 2). COS cells transfected with mutant penta-alanine β_c (^{418}AAAAA^{422}) GM-CSFR and IL-3R α-chain exhibited cell-surface expression of all receptor chains comparable to wild type transfectants (Table 2) but exhibited no high affinity binding of GM-CSF or IL-3 (Figure 2, Table 2). This indicates that substitution of the region 418-422 in β_c has interfered with the ability of β_c to support high affinity GM-CSF and IL-3 binding and suggests that residues in this region may be involved in ligand interaction.

Tyr^{421} is necessary and sufficient for GM-CSF, IL-3 and IL-5 high affinity binding. In order to examine the individual contribution of residues 418-422 of β_c to high affinity GM-CSF, IL-3 and IL-5 binding, each residue in this region was substituted separately with alanine (Figure 1). As above, the ability of individual mutants to mediate high affinity ligand binding was determined by carrying out radioligand saturation binding studies on COS cells expressing mutant β_c together with GM-CSFR and IL-3R α-chains. Results showed that all alanine substitution mutants were cell-surface expressed similarly to wild type β_c (Table 2) and alanine substitution of residues Thr^{419}, Glu^{420} and Asn^{422} had no affect on high affinity ligand binding (Figure 3, Table 2). Alanine substitution of Arg^{418} reduced both GM-CSF and IL-3 high affinity binding by 2-3 fold which is a relatively small effect (Table 2). Interestingly, however, alanine substitution of Tyr^{421} generated a β_c mutant that was unable to support high affinity binding of either GM-CSF or IL-3 (Figure 2, Table 2). The effect of this mutation on high affinity IL-5 binding was also determined by transfecting COS cells with mutant Y421A β_c together with IL5R α-chain. The saturation binding data obtained with radio-iodinated IL-5 exhibited a single class of receptor on Scatchard analysis. Although β_c has a small effect on affinity conversion of IL-5 binding relative to IL-3 and GM-CSF, studies with the mutant Y421A β_c

showed a loss of affinity commensurate with the loss of high affinity IL-5 binding (Table 2). This shows that Tyr^{421} has a similar role in supporting high affinity binding of GM-CSF, IL-3 and IL-5.

The identification of a single residue in a predicted loop of the membrane proximal CRM of β_c that is critical for high affinity GM-CSF, IL-3 and IL-5 binding raised the possibility that this residue alone in the context of a loop might be sufficient to support high affinity binding. To test this notion we re-introduced a tyrosine residue back into a poly-alanine substituted β_c mutant ($^{418}AAAYA^{422}$) (Figure 1). Surprisingly, this mutant was able to support high affinity GM-CSF and IL-3 binding (Figure 4) albeit with a three fold reduced affinity relative to wild type GM-CSF and IL-3 high affinity binding (Table 2). This mutant also restored high affinity binding to IL-5 (Table 2). These findings indicate that the residues adjacent to Tyr^{421} play no direct role in ligand interaction and implicate Tyr^{421} as a key residue in GM-CSF, IL-3 and IL-5 high affinity binding.

Substitution of Tyr^{421} disrupts functional receptor activation.

In order to determine the functional significance of substituting Tyr^{421} of β_c for alanine we measured the induction of signal transducers and activators of transcription (STAT). Productive interaction of GM-CSF, IL-3 and IL-5 with their high affinity receptors leads to signal transduction mediated by β_c (Kitamura *et al.*, 1991b; Kitamura *et al.*, 1992; Takaki *et al.*, 1993). It is now well documented that signalling by β_c activates JAK2 protein-tyrosine kinase (Silvennoinen *et al.*, 1993; Quelle *et al.*, 1994) and subsequently the transcription factor STAT5 is rapidly phosphorylated enabling it to bind to its DNA binding site in the nucleus (Mui *et al.*, 1995; Azam *et al.*, 1995; Gouilleux *et al.*, 1995; Pallard *et al.*, 1995). STAT5 activation therefore represents a relatively immediate response to β_c-mediated receptor signalling. STAT5 has been described in many haemopoietic cell lines including cells of the myeloid and lymphoid lineages (Mui *et al.*, 1995; Azam *et al.*, 1995; Gouilleux *et al.*, 1995; Pallard *et al.*, 1995). In preliminary experiments we investigated Jurkat T-cells transfected with GM-CSFR, IL-3R or IL-5R receptor α-chain together with wild type β_c. A cytokine inducible nuclear DNA binding protein was detected that retarded an oligonucleotide probe containing the STAT5 binding consensus sequence (TTC-N_3-GAA) in an electrophoretic mobility shift assay. This DNA binding protein was inducible after cytokine treatment in cells transfected with α-chain and β_c but not in the absence of β_c, indicating that β_c mediates this ligand-induced process (Figure 5A). Both the unlabelled probe and the related haemopoitein receptor response element (HRRE) (Morella *et al.*, 1995) competed for binding to the radiolabelled β-casein promoter

probe, both of which contain the STAT5 binding consensus sequence. However, a mutant β-casein promoter probe containing mutations in the STAT5 binding consensus and a canonical AP-1 site probe did not compete for binding (Figure 5B). For this reason we refer to this protein as STAT5-like (STAT5-L) as, although it behaves in a similar fashion to STAT5, its true identity has not been determined.

The ability of mutant receptors to activate STAT5-L protein was determined by co-transfecting Jurkat cells with mutant β_c and GM-CSFR or IL-3R α-chain cDNAs. Cell surface expression of receptor subunits was confirmed by flow cytometry using chain specific antibodies and the expression of mutant and wild type β_c was found to be comparable (data not shown). The transfectants were stimulated with either GM-CSF or IL-3 over a range of concentrations and nuclear extracts prepared. The induction of STAT5-L protein was determined by electrophoretic mobility shift assay (EMSA) using the canonical STAT5 DNA binding site from the β-casein promoter as a probe. STAT5-L was activated in response to either GM-CSF or IL-3 in a dose-dependent fashion in the presence of the appropriate α-chain and wild type β_c (Figure 6A & B). Alanine substitution of Tyr^{421} produced a β_c that supported STAT5-L activation in response to GM-CSF with approximately 100-1000 fold reduction in potency (Figure 6A). This reduction in GM-CSF responsiveness with this mutant β_c indicates that Tyr^{421} has a role in GM-CSF induced receptor activation. Significantly, Y421A β_c had a greater effect on IL-3-induced STAT5-L activation producing only very low levels of detectable STAT5-L (Figure 6B) and even at 3 μM IL-3 half maximal activation was not achieved. This suggests that the tyrosine residue is also crucial for the interaction of IL-3 with β_c.

EXAMPLE 2

Generation of antibody antagonists

Monoclonal antibodies can be generated by immunizing with β_c or with a fragment of β_c containing the F'-G' loop or with a peptide containing the F'-G' sequence. After specificity controls demonstrate specific binding to the F'-G' loop the antibodies may be selected for blocking GM-CSF, IL-3 and IL-5 high affinity binding and for blocking GM-CSF, IL-3 and IL-5 stimulation of function. Once an appropriate monoclonal antibody has been identified and shown to block GM-CSF/IL-3/IL-5 high affinity binding and function, smaller fragments may be generated; e.g. $F(ab)_2$, Fab and ultimately Fv. By utilising molecular biology techniques a single chain Fv fragment can be constructed (Hv-Lv). This would be an inhibitory peptide.

EXAMPLE 3

Generation of peptide antagonists

Short peptides of similar sequences to the F'-G' loop may be synthesized that block cytokine interaction with the F'-G' loop of β_c. The reverse is also possible: short peptides of similar sequences to the helix A of GM-CSF/IL-3/IL-5 (this is the region in the cytokines predicted to interact with the F'-G' loop) may also block cytokine interaction.

EXAMPLE 4

Generation of oligonucleotide antagonists

A large pool of randomly synthesized oligonuclotides can be passed through an F'-G' peptide immobilized on a solid matrix (Bock *et al.*, 1992, - which reference is incorporated herein). Following washing, the strongly binding oligonucleotides remain and can then be eluted under different conditions (salt, pH etc). The sequence can then be determined by PCR and tested for inhibition of β_c -mediated function on a real cell system

EXAMPLE 5

Alignment of amino acids of the F-G loops of β_c and gp130

Alignment of the β_c and gp130 has been published. gp130 and β_c contain domains that are related to the fibronectin type III domain that is a characteristic feature of most cytokine receptors. gp130 has 6 of these domains, β_c has four of these domains.

Each domain contains its own F-G loop, thus gp130 has 6 and β_c has 4. Only one of these F-G loops is the important one as far as the present invention is concerned. The important domain is characterised by the presence of i) a conserved pattern of Cys residues, ii) several Trp residues iii) a YXXRV/IR motif, and iv) a WSXWS motif (where X is any amino acid). Tyr ^{421} is postulated to lie within the F-G loop of the fourth domain loop in β_c (F'-G'). The equivalent functionally important tyr in gp130 is postulated to lie within the F-G loop of the second domain.

Table 2 Affinity constants and statistical analysis of the binding of GM-CSF, IL-3 and IL-5 to COS cells transfected with the appropriate α chains and wild type or mutant β_c.

COS cells expressing:			LIGANDS						
			GM-CSF			IL-3		IL-5	
α-chain only[a]			K_d = 2-12 nM			K_d = 20-100 nM		K_d = 1.2-2 nM	
	No. of expts	% FACS +ve cells	No. of sites detected	K_d site 1 (pM ± SE)	K_d site 2 (nM ± SE)	No. of sites detected	K_d site 1 (pM ± SE)	No. of expts	K_d site (pM ± SE)
+Wild type β_c	10	22.8[b]	2 P<0.001[c]	95 ± 17[d]	5.7 ± 1.7	2 P<0.001	141 ± 32[e]	1	644 ± 119[f]
$+^{418}AAAAA^{422}$	4	34.2	1	-[g]	2.9 ± 0.2	1	-	ND[h]	
+R418A	2	47.4	2 P<0.001	223 ± 74	7.0 ± 0.6	2 P<0.001	511 ± 156	ND	
+T419A	2	39.1	2 P<0.001	105 ± 13	13.5 ± 8.0	2 P<0.001	151 ± 55	ND	
+G420A	2	39.8	2 P<0.001	142 ± 34	35.2 ± 9.8	2 P<0.001	196 ± 70	ND	
+Y421A	6	34.2	1	-	2.7 ± 0.3	1	-	2	1858 ± 409
+N422A	2	29.0	2 P<0.001	61 ± 64	7.8 ± 1.0	2 P<0.001	80 ± 53	ND	
$+^{418}AAAYA^{422}$	3	34.5	2 P<0.001	295 ± 99	10.4 ± 1.3	2 P<0.001	113 ± 38	2	710 ± 132

[a] Range of affinities observed for ligand binding to receptor α chain only.

[b] Transfected COS cells positive (+ve) for β_c expression measured by fluoresence-activated cell sorter (FACS) using a cocktail of nine anti-β_c monoclonal antibodies.

[c] Confidence levels expressed as P values for a one site fit versus a two site fit. Where no confidence level is reported two sites could not be fitted.

[d] Binding data from separate experiments (expts) with GM-CSF were co-analysed and the affinity constants are shown ± the standard error (SE) (Munson and Rodbard, 1980).

[e] Analysis of data was performed as above except that given the extreme low affinity of IL-3 binding to IL-3R α chain, a constant value of 50nM was used to allow estimation of high affinity sites (K_d site 1).

[f] Analysis of data was performed as above. A single receptor class was detected with IL-5.

[g] -: no high affinity binding sites were detected.

[h] ND: not done.

Table 3 Alignment of putative F'-G' loops of members of the cytokine receptor superfamily

CONSENSUS	V R X R		W S X W S
		Common receptor subunits	
hβc	V R V R	TSRTGYNGI (SEQ ID No. 1)	W S E W S
hLIFR	F R I R	CSTETFWK (SEQ ID No. 2)	W S K W S
gp130 (CRM1)	F R I R	CMKEDGKGY (SEQ ID No. 3)	W S P W S
hIL-2Rβ	V R V K	PLQGEFTT (SEQ ID No. 4)	W S P W S
hIL-4R/hIL-13R	V R V R V	KTNKLCFDDNKL (SEQ ID No. 5)	W S D W S
hIL-2Rγ	V R S R	FNPLCGSAQH (SEQ ID No. 12)	W S E W S
		Ligand-specific receptor subunits	
hIL-3Rα	V Q I R	ERVYFF (SEQ ID No. 6)	W S A W S
GM-CSFRα	V K I R	AADVR I LN (SEQ ID No. 7)	W S E W S
hIL-5Rα	V Q V R	AAVSSMCREAGL (SEQID No. 8)	W S E W S
		Ligand-specific signalling receptors	
hEPOR	V R A R	MAEPSFGGF (SEQ ID No. 9)	W S A W S
hTPOR (CRM2)	L R A R	LNGPTYQGP (SEQ ID No. 10)	W S S W S
hOBR	V Q V R	CKRLDGLGY (SEQ ID No. 11)	W S N W S

Aromatic hydrophobic residues are underlined

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SEQUENCE LISTING

(1) GENERAL INFORMATION
(iii) NUMBER OF SEQUENCES

(2) INFORMATION FOR SEQ ID NO.1
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1;

Thr Ser Arg Thr Gly Tyr Asn Gly Ile
1 5

(3) INFORMATION FOR SEQ ID NO.2
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2;
Cys Ser Thr Glu Thr Phe Trp Lys
1 5

(4) INFORMATION FOR SEQ ID NO.3
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3;
Cys Met Lys Glu Asp Gly Lys Gly Tyr
1 5

(5) INFORMATION FOR SEQ ID NO.4
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4;
Pro Leu Gln Gly Glu Phe Thr Thr
1 5

(6) INFORMATION FOR SEQ ID NO.5
(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5;

Val Lys Thr Asn Lys Leu Cys Phe Asp Asp Asn Lys Leu
1 5 10

(7) INFORMATION FOR SEQ ID NO.6
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6;

Glu Arg Val Tyr Glu Phe
1 5

(8) INFORMATION FOR SEQ ID NO.7
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7;

Ala Ala Asp Val Arg ILe Leu Asn
1 5

(9) INFORMATION FOR SEQ ID NO.8
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8;

Ala Ala Val Ser Ser Met Cys Arg Glu Ala Gly Leu
1 5 10

(10) INFORMATION FOR SEQ ID NO.9
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9;

Met Ala Glu Pro Ser Phe Gly Gly Phe
1 5

(11) INFORMATION FOR SEQ ID NO.10
(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10;

Leu	Asn	Gly	Pro	Thr	Tyr	Gln	Gly	Pro
1				5				

(12) INFORMATION FOR SEQ ID NO.11
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11;

Cys	Lys	Arg	Leu	Asp	Gly	Leu	Gly	Tyr
1				5				

(13) INFORMATION FOR SEQ ID NO.12
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12;

Phe	Asn	Pro	Leu	Cys	Gly	Ser	Ala	Gln	His
1				5					10

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:-

1. A therapeutic agent capable of binding to the F' - G' loop, of domain 4 of the common β_c chain of the receptor for GM-CSF, IL-3 and IL-5, or to an analogous loop, of an other cytokine receptor the binding of which agent has an antagonist or agonist effect on the respective cytokine or one of the cytokines, said F'-G' or analogous loop not including a WSXWS motif.

2. A therapeutic agent as in claim 1 wherein the cytokine receptor is a common signal transducing receptor.

3. A therapeutic agent as in claim 2 wherein the common signal transducing receptor is selected from the group comprising β_c, gp130, LIFR, IL-2Rβ IL-2Rγ, and IL-4R/IL-13R.

4. A therapeutic agent as in claim 2 wherein the common signal transducing receptor is β_c.

5. A therapeutic agent as in claim 2 wherein said agent has an antagonist or agonist effect on all respective cytokines that bind said common signal transducing receptor.

6. A therapeutic agent as in claim 4 capable of inhibiting the binding between Tyr^{421} of the said F'-G' loop of the common β_c chain and any one of GM-CSF, IL-3 and IL-5.

7. A therapeutic agent as in claim 4 capable of inhibiting the binding between Tyr^{421} of the said F'-G' loop of the common β_c chain and all of GM-CSF, IL-3 and IL-5.

8. A therapeutic agent as in claim 6 capable of binding to Tyr^{421} of the said F'-G' loop of the common β_c chain of the receptor for GM-CSF, IL-3 and IL-5.

9. A therapeutic agent as in claim 1 wherein the F'-G' loop includes a hydrophobic amino acid.

10. A therapeutic agent as in claim 9 wherein the hydrophobic amino acid is a tyrosine.

11. A therapeutical agent as in claim 9 wherein the cytokine receptor is selected from the group comprising GM-CSFRα IL-5Rα, IL-3Rα, EPOR, TPOR, OBR.

12. A therapeutical agent as in claim 9 wherein the cytokine receptor is selected from the group comprising GM-CSFRα IL-5Rα, IL-3Rα.

13. A therapeutic agent as in claim 9 capable of inhibiting the binding of any one cytokine to the respective aromatic hydrophobic amino acids as indicated in table 3 of the respective cytokine receptor.

14. A therapeutic agent as in claim 1 wherein the cytokine receptor is a signalling receptor.

15. A therapeutic agent as in claim 14 wherein the signalling receptor is specific for a single cytokine.

16. A therapeutic agent as in claim 14 wherein the signalling recepter is selected from the group comprising EPOR, TPOR and OBR.

17. A therapeutic agent capable of binding a sequence selected from the group comprising SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11 and SEQ ID NO. 12, of a respective cytokine receptor molecule to thereby act as an antagonist or agonist of corresponding cytokines capable of binding thereto.

18. A therapeutic agent capable of binding a sequence selected from the group comprising SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5 and SEQ ID NO. 12 of a respective cytokine receptor molecule to thereby act as an antagonist or agonist of corresponding cytokines capable of binding thereto.

19. A therapeutic agent capable of binding a sequence selected from the group comprising SEQ ID NO. 6, SEQ ID NO. 7 and SEQ ID NO. 8 of a respective cytokine

receptor molecule to thereby act as an antagonist or agonist of corresponding cytokines capable of binding thereto.

20. A therapeutic agent capable of binding a sequence selected from the group comprising SEQ ID NO. 9, SEQ ID NO. 10 and SEQ ID NO. 11 of a respective cytokine receptor molecule to thereby act as an antagonist or agonist of corresponding cytokines capable of binding thereto.

21. A therapeutic agent capable of binding to the sequence SEQ ID NO. 1, of the β_c receptor molecule to thereby act as an antagonist or agonist of IL-3, IL-5 and GM-CSF.

22. A therapeutic agent as in any one of the preceding claims being an antagonist of the corresponding cytokines.

23. A therapeutic agent as in any one of claims 1 to 21 being an agonist of the corresponding cytokines.

24. A therapeutic agent as in any one of the preceding claims being selected from the group comprising, antibodies or fragments thereof, peptides, oligosaccharides, oligonucleotides and organic and inorganic compounds.

25. A method of isolating a therapeutic agent capable of binding to the F' - G' loop of domain 4 of the common β_c chain of the receptor for GM-CSF, IL-3 and IL-5, or to an analogous loop of another cytokine receptor, said method including the steps of screening candidate molecules for their capacity to bind to the said loop, said F'-G' or analogous loop not including a WSXWS motif.

26. A method of isolating a therapeutic agent as in claim 25 wherein the step of screening candidate molecules for their capacity to bind to said loop, comprises screening for the capacity to bind to a sequence selected from the group comprising SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, SEQ ID NO. 9, SEQ ID NO. 10, SEQ ID NO. 11 and SEQ ID NO. 12.

27. A method of isolating a therapeutic agent as in claim 25 wherein the step of screening candidate molecules for their capacity to bind to said loop, comprises

screening for the capacity to bind to a sequence selected from the group comprising SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5 and SEQ ID NO. 12.

28. A method of isolating a therapeutic agent as in claim 25 wherein the step of screening candidate molecules for their capacity to bind to said loop, comprises screening for the capacity to bind to a sequence selected from the group comprising ID NO. 6, SEQ ID NO. 7 and SEQ ID NO. 8.

29. A method of isolating a therapeutic agent as in claim 25 wherein the step of screening candidate molecules for their capacity to bind to said loop, comprises screening for the capacity to bind to a sequence selected from the group comprising SEQ ID NO. 9, SEQ ID NO. 10, and SEQ ID NO. 11.

30. A method of isolating a therapeutic agent as in claim 25 wherein the step of screening candidate molecules for their capacity to bind to said loop, comprises screening for the capacity to bind to a sequence SEQ ID NO. 1.

31. A method of isolating a therapeutic agent as in claim 25 wherein the agent is an antibody of fragment thereof, said method further including the step of inoculating an animal with a peptide molecule having the F'-G' loop, fusing antibody producing cells with a myeloma cell line and screening for a cell line that produces an antibody reactive with said F'-G' loop, but not with a WSXWS motif, and harvesting antibodies from said cell line, testing for inhibition of high affinity binding, and testing for inhibition or excitation of function.

32. A method of isolating a therapeutic agent as in claim 31 wherein the method further includes making small fragments of antibodies produced by the said cell line capable of binding said the loop.

33. A method of isolating a therapeutic agent as in claim 31 wherein the cell line is a mouse cell line and the method includes the step of humanising the said antibody

fragments by replacing mouse sequences with human sequences in the non-binding regions.

Dated this 18th day of February 1999

MEDVET SCIENCE PTY LTD
By its Patent Attorneys
A.P.T. Patent and Trade Mark Attorneys

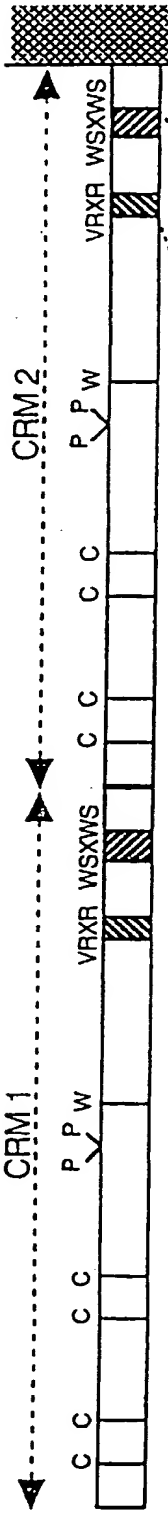

FIGURE 1A

human βc	412-	VRVRTSRTGYNGIWSEWS	-429
human GHR	210-	VRVRSKQRNSGNYGEFS	-226
human PRLR	180-	VQVRCKPDHGYWSAWS	-195

βc mutants

	420
418AAAAA422	VRVRTSAAAAAGIWSEWS
R418A	VRVRTSATGYNGIWSEWS
T419A	VRVRTSRAGYNGIWSEWS
G420A	VRVRTSRTAYNGIWSEWS
Y421A	VRVRTSRTGANGIWSEWS
N422A	VRVRTSRTGYAGIWSEWS
418AAAYA422	VRVRTSAAAYAGIWSEWS

FIGURE 1B

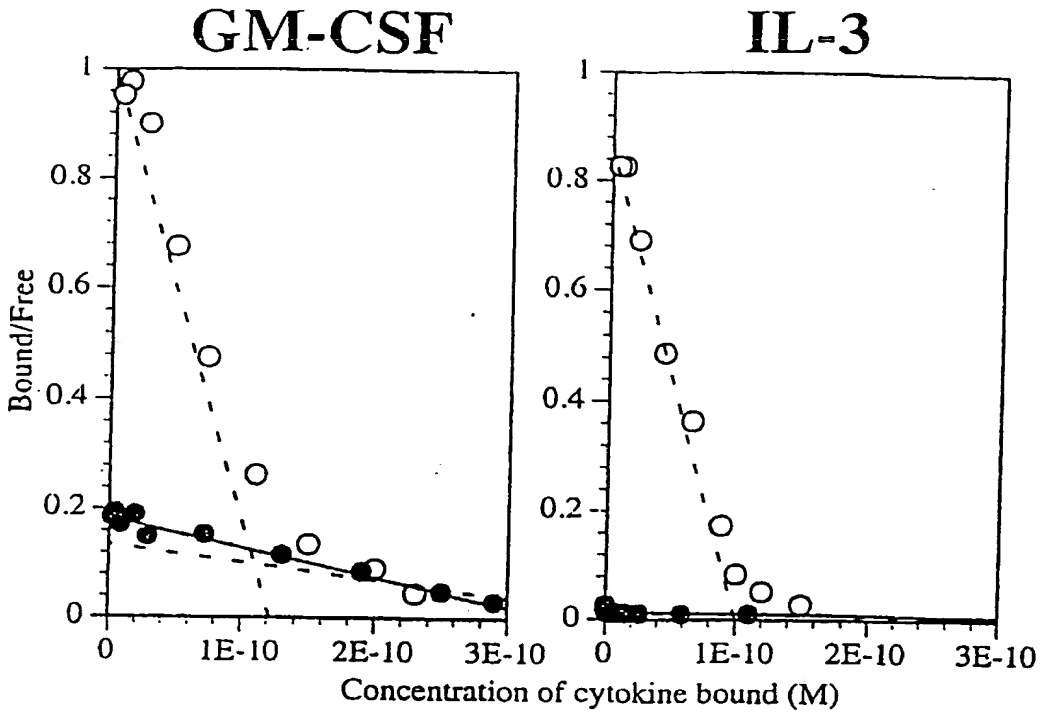
GM-CSF
IL-3
Bound/Free
1
0.8
0.6
0.4
0.2
0
0
1E-10
2E-10
3E-10
Concentration of cytokine bound (M)

FIGURE 2

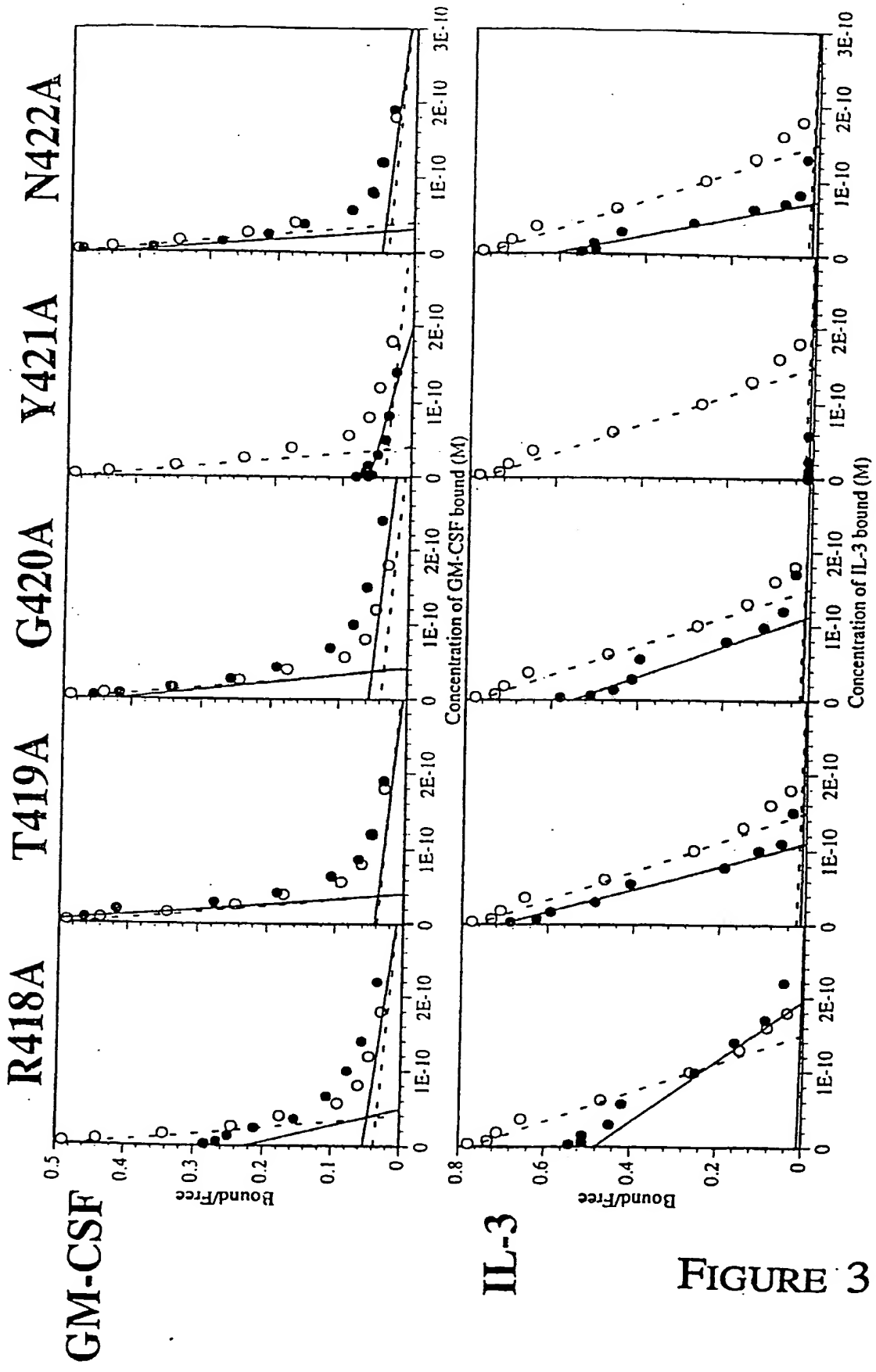
GM-CSF
IL-3
R418A
T419A
G420A
Y421A
N422A
Bound/Free
Concentration of GM-CSF bound (M)
Concentration of IL-3 bound (M)
0
1E-10
2E-10
3E-10
0.1
0.2
0.3
0.4
0.5
0.6
0.8

FIGURE 3

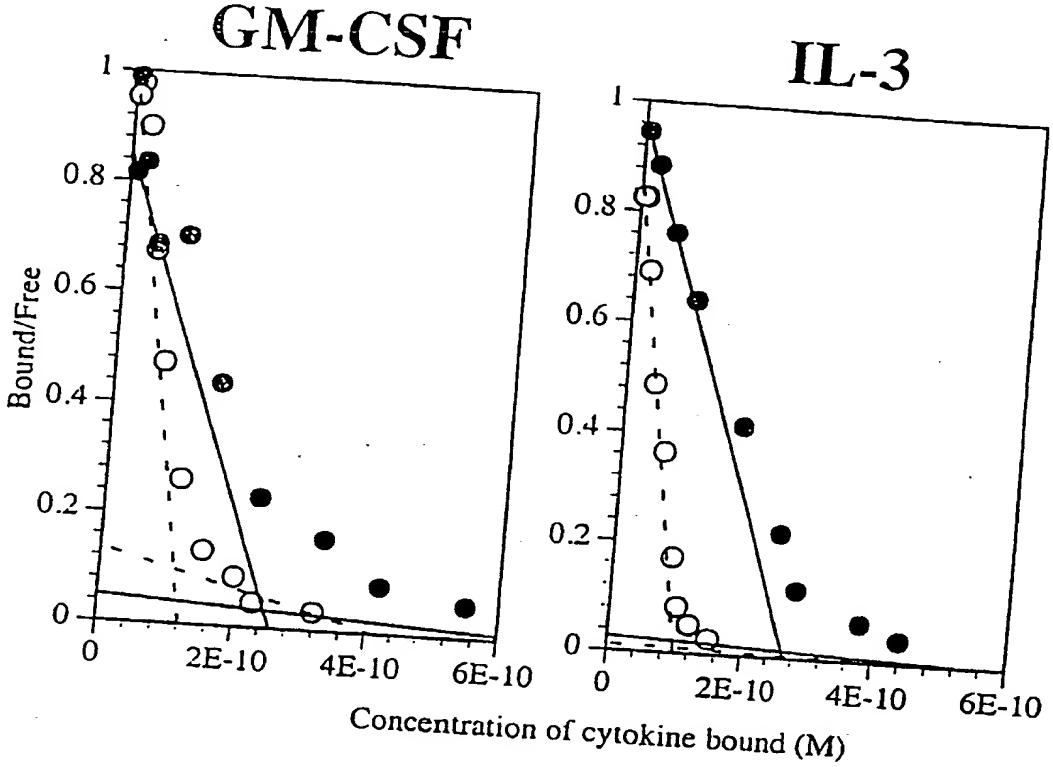
GM-CSF
IL-3
Bound/Free
1
0.8
0.6
0.4
0.2
0
0
2E-10
4E-10
6E-10
Concentration of cytokine bound (M)

FIGURE 4

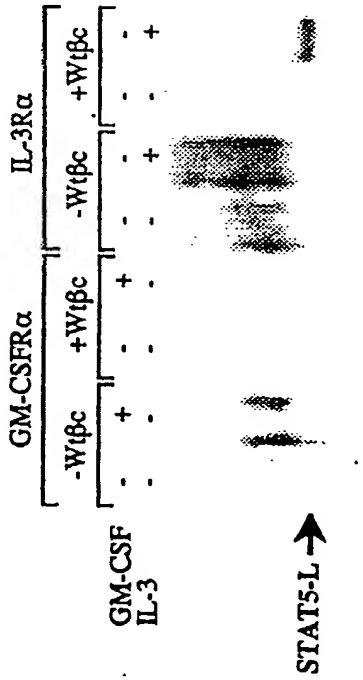

FIGURE 5A

FIGURE 5B

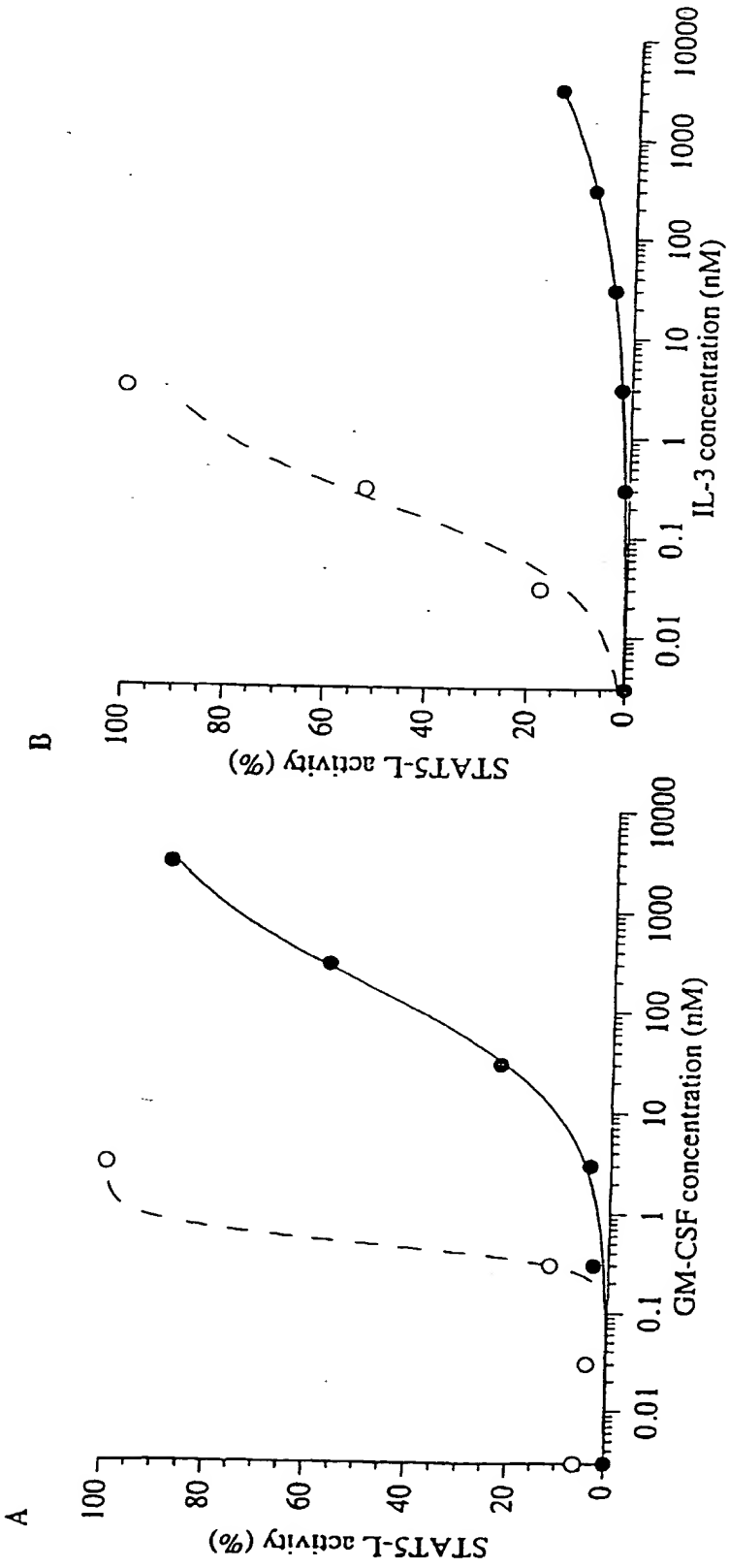

FIGURE 6A

FIGURE 6B

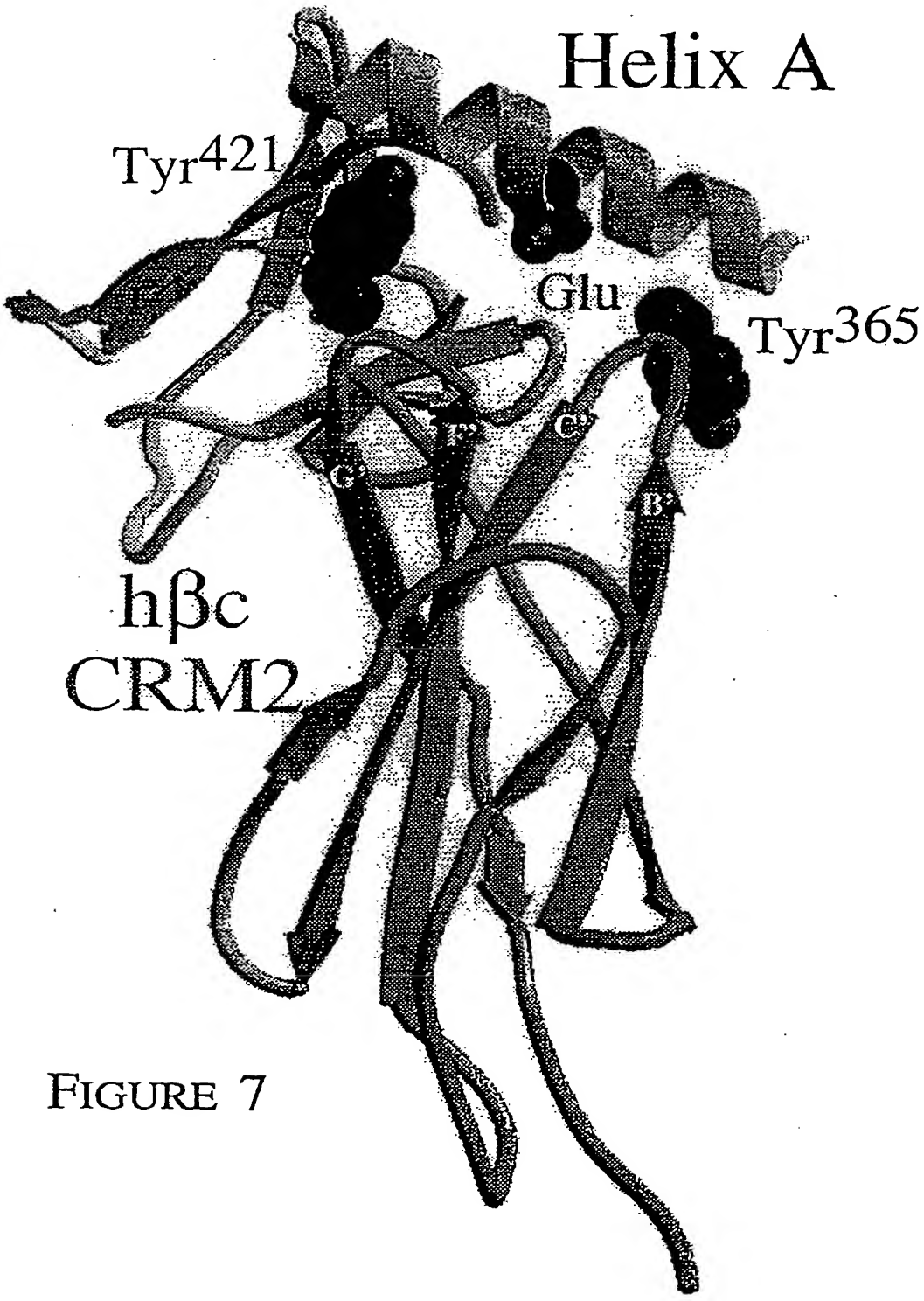

FIGURE 7